JASSA

Journal of Applied Science in Southern Africa
The Journal of the University of Zimbabwe

Volume 6 • Number 1 • 2000 **ISSN 1019–7788**

CONTENTS

JASSA

Journal of Applied Science in Southern Africa
The Journal of the University of Zimbabwe

Vol. 6 No. 1 • 2000
ISSN 1019–7788

CONTENTS

© University of Zimbabwe 2000

Published by University of Zimbabwe Publication
P.O. Box MP203, Mount Pleasant, Harare, Zimbab

Typeset by University of Zimbabwe Publications
Printed by Print Holdings (Pvt.) Ltd., Harare

Pathogenesis, growth and survival characteristics of *Listeria monocytogenes* – a newly emerged food-borne pathogen

H. Nyati

Biology Department, Bindura University of Science Education,
Private Bag 1020, Bindura, Zimbabwe.
Telephone: 263-071-7531/6 Fax: 263-071-7534
E-mail: hnyati@hotmail.com or E-mail: nyati@mailhost.buse.ac.zw

Survival and growth characteristics of *Listeria monocytogenes* isolates were determined in Tryptose Phosphate Broth and in chicken and beef substrates in the period 1993 to 1994. Observed generation times for the Scott A strain (clinical isolate) in Tryptose Phosphate Broth, anaerobically, were 146.2, 21.0, 16.5, 8.2 and 1.6 hours at 0, 3.5, 5.5, 8.0 and 20°C, respectively, compared to 123.4, 23.1, 17.5, 7.5 and 1.5 hours, aerobically. Similar growth rates were observed for strain RMIT 405 (raw chicken isolate), both anaerobically and aerobically in Tryptose Phosphate Broth, chicken and beef substrates. Growth rates were fitted to the square root model with a coefficient of determination (R^2 value) of 98.36 to 99.48 percent. Heating to an internal temperature of 70°C resulted in a 3 to 5 log reduction of all *L. monocytogenes* isolates under study in broth and chicken substrates while a heat treatment to 70°C/2 minutes resulted in a reduction greater than 7 log cycles. Lowering the product pH to 5.0 was effective in inhibiting *L. monocytogenes* growth, whereas a sodium chloride concentration of 2 percent had a negligible effect on growth rates.

Keywords: *Listeria monocytogenes, growth rates, thermal resistance.*

Introduction

Listeriosis was for many years thought to be a zoonotic disease and very little was known until recently about the growth and survival characteristics of its causative agent *Listeria monocytogenes* (Jay, 1992). Refer to Table 1. The role and importance of *L. monocytogenes* (formerly *Bacterium monocytogenes*), a Gram positive asporogenous rod, as an agent of food-borne disease has become of major concern in recent years to the food industry as *L. monocytogenes* is one of the few food-borne pathogens that are capable of growth at refrigeration temperatures under anaerobic, aerobic or microaerophilic conditions. In addition, *L. monocytogenes* is capable of growth in environments of low pH (minimum pH 5.0) and high salinity of a maximum of 10 to 12 percent sodium chloride (Doyle, 1988; Ahamad and Marth, 1989; Rowan, 1989; Sorrells and Enigl, 1990; Presser *et al.*, 1997).

Table 1: Relationship between pathogens, disease caused and year first recognized as pathogens

Food-borne bacterial pathogen	Disease cuased	Year first recognized as pathogen
Salmonella spp.	Salmonellosis	1888
Staphylococcal aureus	Staphylococcal food poisoning	1894
Clostridium botulinum	Botulism	1896
Escherichia coli	Gastroenteritis	1971
Vibrio cholerae	New non- 01 strains	1979
Listeria monocytogenes	Listeriosis	1979

(Jay,1992)

L. monocytogenes is widespread in nature and has been isolated globally from soils, vegetation, silage, meats, vegetables, dairy products and food processing environments such as floors, drains, cleaning aids, processing equipment, ceilings, raw materials storage bins and protective clothing (Schwartz *et al.*, 1988; Ellis, 1989; Farber *et al.*, 1989; Gilbert *et al.*, 1989; Genigeorgis *et al.*, 1989, 1990; Johnson *et al.*, 1990; Kerr *et al.*, 1990; Mafu *et al.*, 1990; Farber, 1991a,b). The clinical manifestations of listeriosis include meningitis, septicaemia, spontaneous abortion, conjuctivitis, oculoglandular listeriosis, cutaneous listeriosis, pneumonic listeriosis and cervicoglandular listeriosis. Symptoms include fever, convulsions, chills, backache, headaches, diarrhoea, vomiting and discoloured urine (Sutherland, 1989). The well categorized risk groups are pregnant women and their foetuses, neonates, the elderly and immuno-compromised adults. Previously-healthy individuals can also become infected during an outbreak with some manifesting minor clinical flu-like symptoms while others become asymptomatic carriers. Listeriosis has been reported to have fatality rates of up to 41 percent but can be treated successfully with antibiotics such as Penicillin and Gentamicin. The minimum infective dose of *L. monocytogenes* in humans has been estimated from the food source to range from $1x10^2$ to $3x10^9$ cells (Farber, 1993).

Several well documented outbreaks of food-borne listeriosis have been reported (Kerr et al., 1988(a, b); Ellis, 1989; Kaczmarski and Jones,1989; Nathoo *et al.*, 1990; Farber and Peterkin, 1991; Russell, 1991). Outbreaks have been associated mainly with dairy products, but raw vegetables and poultry have also been implicated. The first documented outbreak of human listeriosis was in 1981, Nova Scotia, where thirty-nine persons were taken ill after eating commercially prepared coleslaw, and the fatality rate was 41 percent. The source was traced to cabbage from a field that had been fertilized with manure from infected sheep. The cabbages had been held in cold storage for an extended period prior to processing allowing the listeriae to multiply (Ellis, 1989). Other documented outbreaks are listed in Table 2.

Table 2: Reported Listeriosis outbreaks and incidents.

Area/Year	Source of Listeriosis	Number of people affected	Mortality rate (%)
Massachusetts, 1983	pasteurized milk	49 (7 perinatal)	12
Los Angeles,1985	soft cheese	142 (mother/infant)	30
United Kingdom,1988	cook-chill chicken	1 (materno-foetal)	miscarriage
United Kingdom,1989	chicken nuggets	2 (previously healthy adults)	0
Australia,1990	cheese	2 (mother/infant)	0
Zimbabwe,1990	unknown	1 (newborn)	100

Listeriosis is now a clinically notifiable condition in Victoria, Australia, which should be reported in writing to the Health Department by a laboratory or doctor making the diagnosis (Carnie, 1991). In addition the USDA (United States Department of Agriculture) has set a zero tolerance limit (absence in a 25g sample) for *L. monocytogenes* in ready-to-eat products (Mitchell, 1988; Bryan, 1990; Anon, AQIS, 1992; Farber, 1993). One of our recommendations is that if Southern Africa is to have a thriving food industry it must be able to meet the microbiological standards required by the global market.

In this paper, growth and survival characteristics of *L.monocytogenes* in chicken, beef, and Tryptose Phosphate Broth are discussed in detail.

Materials and Methods

Source of cultures

L. monocytogenes strains were isolated from raw chicken breast samples, pork, lamb, veal, (Melbourne, Australia) and a clinical isolate, Scott A, was obtained from the University of Queensland, Australia.

Maintenance of cultures

Cultures were maintained on Tryptone Soya Agar slants at 4°C for the duration of the investigation. All culture media used were obtained from Oxoid, Basingstoke, UK.

Confirmation of L. monocytogenes isolates

Listeria species were presumptively identified by their ability to hydrolyse aesculin in Palcam agar to black iron phenolic compounds, producing black zones around the colonies. The colonies appear as slightly raised grey-green colonies with a central depression after incubation at 30°C for 48 hours. In addition, *Listeria* species are catalase positive, oxidase negative, Gram positive rods. Oxidase test paper strips (Disposable Products Pty Ltd, South Australia) impregnated with N,N,N,N-tetramethyl-p-phenylene diamine dihydrochloride which changes colour from a

light pink to blue in the presence of oxidase was used to test for oxidase production. The production of catalase was determined by effervescence on contact with 3 percent hydrogen peroxide. The organism showed a slight rotating or tumbling motility when examined by wet mount and also showed umbrella motility in S.I.M. Motility Medium when grown at 20 to 25°C.

Biochemical tests were carried out using a Listeria Micro ID kit (Organon Teknika, Durham, N.C., USA) to identify *Listeria* isolates to species level. The kit tests for acetoin production (Voges Proskauer), nitrate reduction, phenylalanine deaminase, hydrogen sulphide production, indole production, ornithine decarboxylase, lysine decarboxylase, malonate utilisation, urease, esculin hydrolysis, β-galactosidase, xylose, rhamnose, mannitol and sorbitol fermentation. *L. monocytogenes* is identified by its failure to utilise xylose and its ability to utilise rhamnose. For the final confirmation, CAMP tests were carried out on sheep blood agar plates with cultures of *Staphylococcus aureus* (CIP 5710 / NCTC7428) and *Rhodococcus equi* (NTCT162, University of Queensland) streaked in parallel in one direction. *L. monocytogenes* showed enhanced β-haemolytic activity in the vicinity of the *S. aureus* streak, while *L. innocua* is non-haemolytic and *L. ivanovii* haemolysis is enhanced near the *R. equi* streak line.

Thermal inactivation of L. monocytogenes in chicken breast samples and Tryptose Phosphate Broth

Raw skinless chicken breasts were inoculated internally with 10^3 to 10^4 CFU/g of *L. monocytogenes* stationary phase cells (22 to 24-hour cultures in Tryptose Phosphate Broth at 30°C). Stationary phase cultures (10^9 CFU/mL) were diluted to 10^7 CFU/mL in buffered peptone water (0.1 percent), and 0.1 mL injected into chicken breast samples (150g) to achieve a final 5×10^3 CFU/g of *L. monocytogenes*. Inoculated samples in barrier bags (W.R. Grace, Australia) were then vacuum packed in a Vacuumatic 282 (Cryovac) vacuum packer, processed in a water bath at 80°C to an internal temperature of 70°C or 70°C/2min before chilling in ice to 3°C, and stored at 3°C or 8°C for 5 weeks.

Duplicate samples were tested for the presence of *L. monocytogenes* by a two-stage enrichment method (United States Department of Agriculture). For primary enrichment, a sample (25g) was blended with a fixed volume (225mL) of UVM 1 (University of Vermont Listeria Primary Enrichment Broth) and incubated at 30°C for 24 hours. From the primary enrichment, an aliquot (0.1mL) was then sub-cultured into secondary enrichment broth (UVM II, 10mL). The secondary enrichment broth was incubated for a further 24 hours at 30°C before streaking onto Palcam agar and incubation for 48 hours at 30°C (Anon, Oxoid, 1990).

The thermal resistance of seven *L. monocytogenes* strains was also determined in Tryptose Phosphate Broth. Stationary phase cultures (22 to 24 hours, 20mL) were heated from 20°C to 70°C and 70°C/2min, respectively, in Universal bottles immersed to just below the cap in an 80°C water bath. The cultures were then chilled immediately in ice and survivors enumerated on Palcam Agar spread plates. Temperatures were monitored in uninoculated broth.

Determination of the growth rates of L. monocytogenes in vacuum packaged pasteurized chicken breast and beef sirloin

Inoculum preparation

Single colonies from a Tryptone Soya Agar culture were inoculated into Tryptose Phosphate Broth (20mL) and incubated at 10°C for 72 hours to an optical density of 30 to 70 (3×10^8 to 7×10^8 CFU/mL log phase) as determined on a nephelometer (Corning, Unigalvo, Essex, UK). Broth cultures were centrifuged at 6 000 rpm for 15 minutes (Beckman J2-21M/E) and the pellet re-suspended in isotonic saline (0.8 percent). Cultures were further diluted in saline to the range 10^5 to 10^6 CFU/mL before inoculation.

Determination of growth rates

Chicken breast samples (10 g) and beef sirloin were heated to 70°C/2 minutes in vacuum pouches (Cryovac RX673) and chilled to <7°C before internal inoculation by microsyringe with aliquots (0.1 mL each) of a 10^5 to 10^6 CFU/mL Scott A or RMIT 405 *L. monocytogenes* culture. Samples were vacuum-sealed and stored at 3, 8, 10 and 15°C, and *L. monocytogenes* levels were determined at appropriate intervals by addition of an accurately measured volume (99 mL) of buffered peptone water (0.1 percent) to each sample (10 g) before plating out of aliquots (0.1 mL) of appropriate dilutions on Palcam agar plates.

Generation times were calculated according to the formula:

$$k = \frac{\log X_t - \log X_o}{(0.301)t}$$

where X_o = population at beginning of log phase
X_t = population at peak of log phase
t = time from X_o to X_t
k = growth rate
and $1/k$ = generation time (hours)

(Petran and Zottola, 1989)

Growth rates in Tryptose Phosphate Broth

Single colonies from a tryptone soya agar culture were inoculated into Tryptose Phosphate Broth (20 mL) in a nephelometer tube and incubated at 10°C for 72 hours to an optical density of 30 to 60 nephelometer units equivalent to 3×10^8 to 6×10^8 CFU/mL in the logarithmic growth phase.

The culture was diluted in a 0.1 percent buffered peptone water to 3×10^7 to 6×10^7 CFU/mL and a small volume (0.1 mL) was inoculated with a micropipette into Tryptose Phosphate Broth (20 mL) to give an inoculum level of 1.5×10^5 to 3×10^5 CFU/mL. The culture was then incubated at 0 to 25°C, aerobically in a temperature gradient incubator (TN3 - Toyo Kagaku Sangyo Co. Ltd, Japan) at twenty shakes per minute. To achieve anaerobic conditions, Tryptose Phosphate Broth tubes were covered immediately after autoclaving at 121°C/15 min with sterile liquid paraffin (6 mL) autoclaved at 115°C/30 minutes to exclude air. The inoculum was introduced below the paraffin layer with a micropipette and tubes incubated at 0 to 25°C in a

temperature gradient incubator without shaking. Optical densities were determined at appropriate intervals by nephelometry and converted from a standard curve to cell numbers in colony forming units/mL.

The square root of the growth rate was plotted against the temperature of incubation, regression analysis carried out on a MINITAB statistical software package and a line of best fit plotted. Observed values were then compared to those falling on the line of best fit in the square root model (Ratkowsky *et al.*, 1983; McMeekin and Olley, 1986; Adair *et al.*, 1989; Gould, 1989; Grau and Vanderlinde, 1990; Ross and McMeekin, 1991; Grau and Vanderlinde, 1993).

Effect of pH on L. monocytogenes growth

Chicken breast samples (total mass 600 g of 10 g portions each) were marinated in lemon juice (45 mL, pH 2.2) for 1 hour at 3°C, lowering the pH from 5.6 to anywhere in the range 4.9 to 5.0. The pH of representative samples (6 g blended with deionised water (54 mL)) was determined on a Metrohm Herisau E520 glass electrode pH-meter. Samples were then thermally processed in barrier bags to an internal temperature of 70°C/2 minutes in a water bath at 80°C, chilled to <7°C, inoculated internally to a listerial population of 10^3 CFU/g, vacuum sealed, and stored at 15, 10 and 3°C, respectively. *L. monocytogenes* populations were then determined at appropriate intervals on Palcam agar. For an assessment of the correlation between behaviour in low pH food and broth models, Tryptose Phosphate Broth was adjusted from pH 7.3 to 5.6, 5.0 and 4.4 with citric acid *and L. monocytogenes* cell numbers determined by nephelometry and plate counts.

Effect of sodium chloride concentration on L. monocytogenes growth

Sodium chloride (2 percent, 3 percent and 4 percent w/w total concentration) was added to individual chicken breast samples (10 g each) in barrier pouches. Samples were then thermally processed to an internal temperature of 70°C/2 minutes, inoculated internally to 10^3CFU/g, vacuum sealed and incubated at 3, 8, 10 and 15°C, respectively. *L. monocytogenes* populations were then determined at appropriate intervals on Palcam agar spread plates.

Results

Survival of L. monocytogenes during thermal processing

L. monocytogenes was not recovered from chicken breasts inoculated internally with 3×10^3 to 5×10^3 CFU/g of Scott A or RMIT 405 and subjected to thermal processing to an internal temperature of 70°C or 70°C/2 minutes. There was also no growth to detectable levels of *L. monocytogenes* during a storage period of 5 weeks at 3 and 8°C, respectively. This was in spite of the fact that the inoculation rate was higher than that encountered in non-inoculated samples which contained less than 10^2 CFU/g *L. monocytogenes*. In addition, a heat treatment from 20°C to 70°C in Tryptose Phosphate Broth resulted in a reduction of the viable count by 3.1 to 4.7 logs in the strains under study, while a heat treatment to 70°C/2 min resulted in a reduction of the viable count by greater than 7 log cycles (Table 3). Results were consistent with a D_{70} value of 0.14 to 0.27 minutes in homogenates of chicken and beef steak reported by Gaze *et al.*, (1989) and Ben-Embarek and Huss (1993).

Table 3: Thermal resistance of *L. monocytogenes* in Tryptose Phosphate Broth.

Strain	Log reduction in 22 hour cultures after heat-treatment to	
	70°C	70°C/ 2min
Cheese isolate (UQM 3174)	4.7	$>10^7$
Pork isolate (RMIT 401)	4.1	$>10^7$
Lamb isolate (RMIT 402)	4.3	$>10^7$
Veal isolate (RMIT 403)	5.1	$>10^7$
Chicken isolate I (RMIT 404)	4.1	$>10^7$
Chicken isolate II (RMIT 405)	4.4	$>10^7$
Clinical isolate (Scott A)	3.1	$>10^7$

Table 4: Growth rates of *L. monocytogenes* (Scott A and RMIT 405), aerobically and anaerobically, in Tryptose Phosphate Broth.

	Scott A*		RMIT 405*	
Temp (°C)	Anaerobic	Aerobic	Anaerobic	Aerobic
0	146.2	123.4	187.5	189.7
3.5	21.0	23.1	20.5	29.1
5.5	16.5	17.5	12.6	15.0
6.5	10.1	15.0	10.5	12.0
8.0	8.2	7.5	7.3	8.7
9.5	5.8	6.3	5.5	6.9
10.5	5.2	6.0	4.8	5.3
11.5	4.3	4.8	4.3	4.6
13.5	3.6	–	3.4	3.3
14.5	2.7	–	2.8	2.5
15.0	2.5	2.5	2.6	2.5
16.0	2.5	2.4	2.3	1.9
16.5	2.4	2.1	2.2	1.9
17.0	2.1	1.9	2.0	1.8
17.5	2.0	1.8	1.9	1.7
18.0	1.9	1.7	1.8	1.7
18.5	1.8	–	1.8	1.7
19.0	1.7	1.6	1.7	1.6
19.5	1.7	1.6	1.6	1.6
20.0	1.6	1.5	1.4	1.4
20.5	1.6	1.3	1.4	1.3
21.0	1.5	1.3	1.3	–
22.5	1.2	1.2	1.1	1.2
23.0	1.1	–	1.1	–
23.5	1.1	1.1	1.0	1.1
24.0	1.0	1.0	1.0	1.1
25.0	1.0	1.0	1.0	1.0

Scott A*, RMIT 405* – Observed generation time (hours)

Table 5: Growth rates of *L. monocytogenes*, anaerobically, in pasteurized chicken breast and beef sirloin samples.

Isolate	Substrate	Temp (°C)	Generation time (hr)
Scott A	Chicken	0	165.0
	breast	2.5	30.0
		4.5	19.5
		8.0	9.6
		10.0	6.9
		15.0	2.7
RMIT 405	Chicken	0	114.3
	breast	2.5	30.0
		4.5	19.5
		8.0	7.2
		10.0	6.3
		15.0	2.7
Scott A	Beef	0	121.0
	sirloin	2.5	31.5
		4.5	21.8
		8.0	10.2
		15.0	3.5

L. monocytogenes growth rates aerobically and anaerobically in broth models and in chicken and beef substrates

Although *L. monocytogenes* is capable of growth at refrigeration temperatures, low storage temperatures were found to be an effective means of controlling *Listeria* growth in foods, with generation times of 114.3 to 189 hours at 0°C and 30 hours at 2.5°C, compared with a generation time of one hour at 25°C (Table 4 and 5). Growth rates were successfully fitted to the square root model, with a coefficient of determination (R^2 value) of 98.36 to 99.48 percent in broth, chicken and beef substrates (Figures 1 to 6). The percentage difference between observed and expected generation times from 5.5°C to 25°C in broth models were from 0.4 to 13.8 percent with lower temperatures showing greater deviation of up to 55.3 percent from expected results at 0°C. In addition, on comparing predicted generation times with those observed in chicken breast and beef sirloin samples of unmodified pH, the percentage differences at 4.5 to 15°C were from 0.5 to 25.7 percent but as high as 38 to 193 percent at 0 to 2.5°C. Furthermore, although both *L. monocytogenes* strains were found to thrive equally well under aerobic and anaerobic conditions at 13.5 to 25°C, more rapid growth rates were observed under anaerobic conditions at lower temperatures, with the exception of Scott A at 0°C (Table 4).

Table 6: Effect of pH (citric acid) on *L. monocytogenes* anaerobic growth rates in chicken breast samples and Tryptose Phosphate Broth.

		Chicken breast				Tryptose Phosphate Broth	
		Scott A		RMIT 405		Scott A	RMIT 405
Temp (°C)	pH	G*	L**	G**	L*	G*	G*
3	4.4	NG	NG	NG	NG	NG	NG
	4.9-5.1	NG	NG	NG	NG	NG	NG
	5.2-5.4	36.6	100	NG	NG	NG	NG
	5.6	30	50.0	30.0	60.0	78.0	63.0
8	4.9 - 5.1	11.4	40.0	15.0	>63	–	–
	5.6	9.6	0	7.2	0	–	25.5
10	4.9 - 5.1	10.5	40	9.6	>63	NG	NG
	5.2 - 5.4	9.0	12	9.0	20	NG	NG
	5.6	6.9	0	6.3	0	26.1	18.1
15	4.9 - 5.1	9.0	16.0	7.8	16.0	NG	NG
	5.2 - 5.4	3.3	7.0	3.3	7.0	NG	NG
	5.6	2.7	0	2.7	0	7.8	6.8

G* = Generation time (hours), L** = Lag time (hours) and NG= No growth

Sodium chloride, at levels normally applied in processing had a negligible effect on *L. monocytogenes* growth rates, with generation times of 7.8 versus 7.2 hours at 8°C and 25.2 versus 30.0 hours at 3°C in salted (2 percent) and unsalted chicken breast samples, respectively. However, acidic conditions (pH 5.0 due to citric acid dissociation) were strongly inhibitory to growth, particularly at low temperatures, with no growth observed for more than 28 days in chicken breast samples and Tryptose Phosphate Broth at 3°C (Table 6). The toxicity of organic acids such as citric acid (reported dissociation constant, K_a of 8.12×10^{-4}, pK_a 3.09) is not primarily caused by hydrogen ion concentration but by the action of undissociated molecule (Ahamad and Marth, 1989; Presser *et al.*, 1997) .The mechanism of activity has been related to interference with:

- permeability of the microbial cell membrane
- substrate transport
- oxidative phosphorylation and
- the release of hydrogen ions on adsorption of the anion by the cell wall.

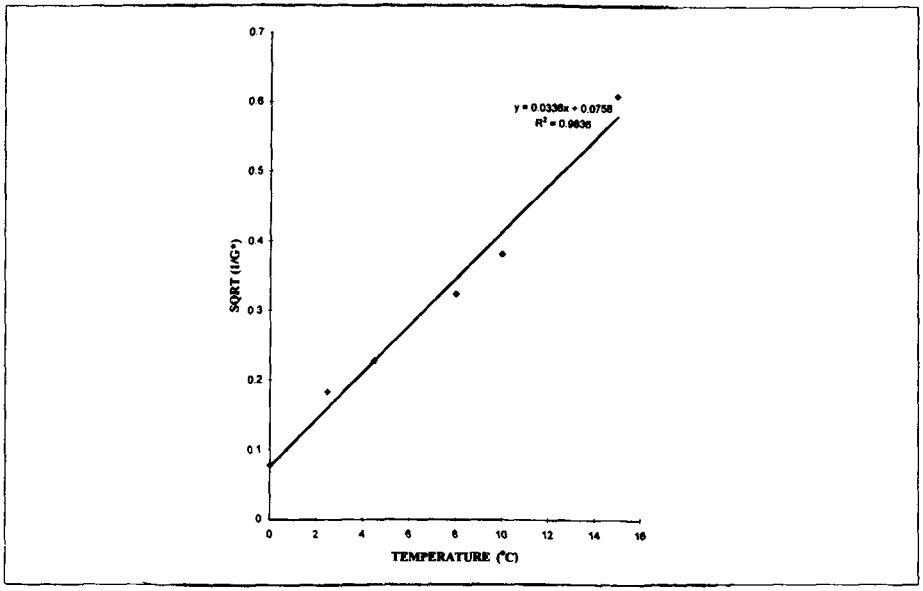

Figure 5: Square root model for *L. monocytogenes* (Scott A) growth rates, anaerobically, in pasteurized chicken breast samples. (G*= generation time in hours).

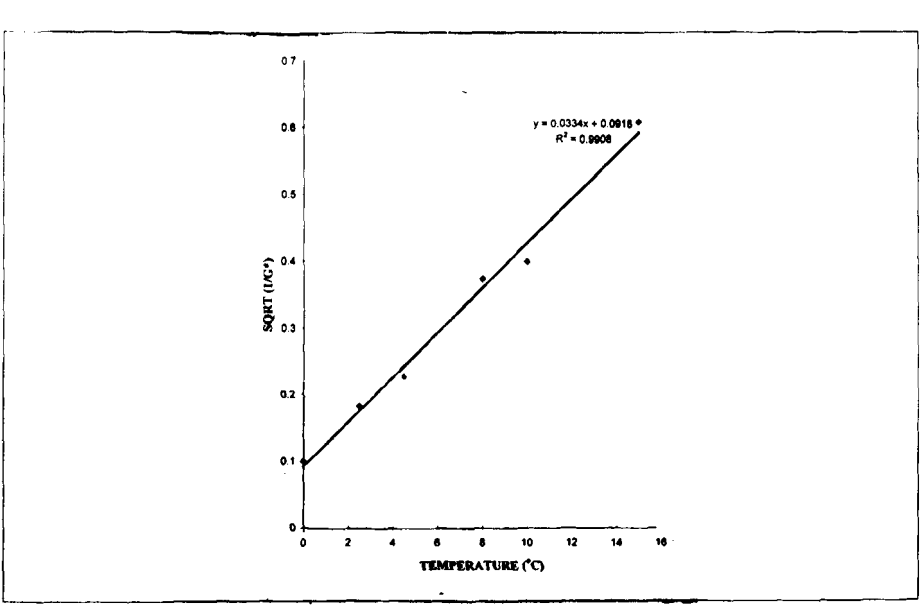

Figure 6: Square root model plot for *L. monocytogenes* (RMIT 405) growth rates, anaerobically, in pasteurized chicken breast samples. (G*= generation time in hours).

Discussion

Thermal processes to an internal temperature of 70°C / 2 min are listeriocidal, with a high safety margin of > 7 log reductions. However, it is recommended that processors demonstrate an even heat distribution within processing equipment, to ensure that the intended thermal process is delivered. Time-temperature recorders should also be used to monitor temperatures during production.

The growth of any survivors can be minimized by maintaining storage temperatures at 0 to 3°C. Storage temperatures may be monitored by electronic temperature recording devices providing a print-out of the complete temperature history of the product or biochemical time-temperature integrators. Biochemical time-temperature integrators may be strips attached to individual packages, whose function is based on enzyme-substrate reactions and change colour when the product is considered to be improperly stored (McMeekin and Olley, 1986).

Predictive growth modelling is a useful tool in estimating cumulative microbial growth, given the time-temperature history of a product, although the accuracy of the square root model declined in the lower temperature range of 0 to 5°C. The psychrotrophic nature of *L. monocytogenes* may be the reason for the more rapid growth rates observed at these temperatures than was predicted by the square root model. An under-estimation of the growth rate would then result in storage conditions that allow growth of *L. monocytogenes* to infective levels. Adair *et al.*, (1989) also made the observation that the square root model predictions show a systematic divergence from the observed values as the lower temperature limits for growth are approached. In addition, broth models were found to be inaccurate indicators of microbial behaviour in low pH food systems with generation times of 9 hours in chicken breast and no growth in broth at a pH of 5.2 and a temperature of 10°C. This could be attributed to differences in the initial pH of broth (7.3) and chicken (5.6) and the subsequent use of larger volumes of citric acid to lower the pH of broth models.

To ensure a listeriocidal thermal process it is recommended that stipulated processing times and temperatures should be strictly adhered to and closely monitored. Chilled storage at temperatures of ≤3°C will suppress the growth of any survivors, and low pH formulations (pH less than 5.0) may be employed together with low temperature storage (hurdle technology) to inhibit the growth of *L. monocytogenes*, thus enhancing the safety of ready-to-eat chilled foods.

ACKNOWLEDGEMENTS

The author would like to thank the Australian International Development Assistance Bureau and the Royal Melbourne Institute of Technology for financial and technical assistance rendered. Elisabeth Gorczyca, thanks for the guidance and advice given.

REFERENCES

ADAIR, C., KILSBY, D.C. AND WHITTALL, P.T. 1989 Comparison of the Schoolfield (non-linear Arrhenius) model and the square root model for predicting bacterial growth in foods. *Food Microbiology*, **16**: 7–18.

AHAMAD, N AND MARTH, E. 1989 Behaviour of *Listeria monocytogenes* at 7, 13, 21 and 35°C in Tryptose broth acidified with acetic, citric or lactic acid. *Journal of Food Protection*, **52**: 688–695.

ANON, OXOID. 1990 The Oxoid Manual of Culture Media, 6th edn. Basingstoke, UK.

ANON, AQIS. 1992 Australian Quarantine and Inspection Service. Code of Hygienic Practice for heat-treated refrigerated foods packaged for extended shelf life.

BEN-EMBAREK, P. K. AND HUSS, H. H. 1993 Heat resistance of *Listeria monocytogenes* in vacuum packaged pasteurised fish fillets. *International Journal of Food Microbiology*, **20(2)**: 85–95.

BRYAN, F. 1990 Application of HACCP to ready to eat chilled foods. *Food Technology*, July: 70–77.

CARNIE, J. 1991 *Listeria monocytogenes* - Legislative requirements under the new Health (Infectious Diseases) regulations 1990. *Food Australia*, **43(3)**: 107–109.

DOYLE, M. P. 1988 Effect of environmental and processing conditions on *L.monocytogenes*. *Food Technology*, April:169–171.

ELLIS, R. F. 1989 Control of *Listeria* in processed food plants. *Food Processing*, **50 (11)**: 201–203.

FARBER, J. M., SANDERS, G. W AND JOHNSTON, M. A. 1989 A survey of various foods for the presence of *Listeria* species. *Journal of Food Protection*, **52(7)**: 456–458.

FARBER, J. M. 1991(a) Microbiological aspects of modified atmosphere packaging technology — a review. *Journal of Food Protection*, **54(1)** :58–70.

FARBER, J. M. 1991(b) *Listeria monocytogenes* in fish products. *Journal of Food Protection*, **54(12)**: 922–924.

FARBER, J. M. AND PETERKIN, P. I. 1991 *Listeria monocytogenes* — A food-borne pathogen. *Microbiological Reviews*, **55(3)**:476–511.

FARBER, J. M. 1993 Current research on *Listeria monocytogenes* in foods — an overview. *Journal of Food Protection*, **56(7)**: 640–643.

GAZE, J., BROWN, G. D., GASKELL, D. E. AND BANKS, J. G. 1989 Heat resistance of *Listeria monocytogenes* in homogenates of chicken, beef, steak and carrot. *Food Microbiology*, **6** : 251–259.

GENIGEORGIS, C., DUTULESCU, D. AND GARAZAYBAL, J. 1989 Prevalence of *Listeria* spp., in poultry meat at the supermarket and slaughterhouse level. *Journal of Food Protection*, **52(9)**: 618–624.

GENIGEORGIS, C., OANCA, P. AND DUTULESCU, D. 1990 Prevalence of *Listeria* spp., in Turkey meat at the supermarket and slaughterhouse level. *Journal of Food Protection.* **53(4)** :282–288.

GILBERT, R. J., MILLER, K. L. AND ROBERTS, D. 1989 *Listeria monocytogenes* and chilled foods. *The Lancet*, **1**:383–384.

GOULD, G. 1989 Predictive mathematical modelling of microbial growth and survival in foods. *Food Science and Technology Today*, **3(2)**: 89–92.

GRAU, F. H AND VANDERLINDE, P. B. 1990 Growth of *Listeria monocytogenes* on vacuum packaged beef. *Journal of Food Protection*, **53(9)**: 739–741.

GRAU, F AND VANDERLINDE, P. 1993 Aerobic growth of *Listeria monocytogenes* on beef lean and fatty tissue: Equations describing the effects of temperature and pH. *Journal of Food Protection*, **56**:96–101.

JAY, J. M. 1992 Microbiological Food Safety. *Critical Reviews in Food Science and Nutrition*, **31(3)**:177–190.

JOHNSON, J., DOYLE, M. P. AND CASSENS, R. G. 1990 *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products - a review. *Journal of Food Protection*, **53(1)** :81–91.

KACZMARSKI, E. B. AND JONES, D. M. 1989 Listeriosis and ready cooked chicken. *The Lancet*, **1**: 549.

KERR, K.G., DEALLER, S. AND LACEY, R.W. 1988(a) Martenofetal listeriosis from cook-chill and refrigerated foods. *The Lancet*, **2(8620)**: 1133.

KERR, K. G., DEALLER, S. AND LACEY, R. W. 1988(b) *Listeria* in cook-chill food. *The Lancet*, July: 37–38.

KERR, K., ROTOWA, N., HAWKEY, P. AND LACEY, R. 1990 Incidence of *Listeria* spp. in precooked, chilled chicken products as determined by culture and enzyme linked immuno assay (ELISA). *Journal of Food Protection*, **53(7)**: 606–607.

MAFU, A., ROY, D., GOULET, J. AND MAGNY, P. 1990 Attachment of *Listeria monocytogenes* to stainless steel, glass, propylene and rubber surfaces after short contact times. *Journal of Food Protection*, **53(9)**: 742–746.

MCMEEKIN, T. A AND OLLEY, J. 1986 *Predictive Microbiology. Food Technology Australia*, **38 (8)**: 331–334.

MITCHELL, P. 1988 Refrigerated foods trend draws FDA and USDA vigilance. *Food Processing*, **49(7)**: 8–10

NATHOO, K. J, MASON, P. R., CHIMBIRA, T. H. K AND THE PUERPERAL SEPSIS STUDY GROUP. 1990 Neonatal septicaemia in Harare Hospital; aetiology and risk factors, **36(6)**: 150–156.

PETRAN, R AND ZOTTOLA, E. 1989 *Central African Journal of Medicine* A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. *Journal of Food Science*, **54(2)**: 458–460.

PRESSER, K. A., RATKOWSKY, D. A. AND ROSS, T. 1997 Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. *Applied and Environmental Microbiology*, **63**: 2355–2360.

RATKOWSKY, D. A., LOWRY, R. K., MCMEEKIN, T. A., STOKES, A. N. AND CHANDLER, R. E. 1983 Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *Journal of Bacteriology*, **154(3)**: 1222–1226.

ROSS, T. AND MCMEEKIN, T. A. 1991 Principles and potential of predictive microbiology – Application of a square root model for *Staphylococcus aureus* growth in seafood products. *Food Australia*, **43**: 202–207.

ROWAN, C. 1989 *Listeria* hysteria-or is it? Food manufacture, March: 42–46.

RUSSELL, E. G. 1991 Clinical cases of listeriosis. *Food Australia*, **43(3)**: 104–107.

SCHWARTZ, B., CIESILSKI, C. A., BROOME, C. V., GAVENTA, S., BROWN, G. R., GELLIN, B. G., HIGHTOWER, A. W., MASCOLA, L. AND THE LISTERIOSIS STUDY GROUP. 1988 Association of sporadic listeriosis with consumption of uncooked hot dogs and undercooked chicken. *The Lancet*, **2**: 779–782.

SORRELLS, K AND ENIGL, D. 1990 Effect of pH, acidulant, sodium chloride and temperature on the growth of *Listeria monocytogenes*. *Journal of Food Safety*, **11**: 31–37.

SUTHERLAND, P. S. 1989 *Listeria monocytogenes*. Food borne microorganisms of Public Health Significance (4th edn). AIFST NSW Food Microbiology Group, Sydney.

This work is licensed under a
Creative Commons
Attribution – NonCommercial - NoDerivs 3.0 License.

To view a copy of the license please see:
http://creativecommons.org/licenses/by-nc-nd/3.0/

This is a download from the BLDS Digital Library on OpenDocs
http://opendocs.ids.ac.uk/opendocs/

Institute of Development Studies